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INTERNAL AND EXTERNAL ANATOMY OF THE ABDOMINAL DISC OF *ATELOPUS* (BUFONIDAE) LARVAE

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Resumen
El disco abdominal de *Atelopus* es una estructura compleja que presenta tres regiones bien definidas así como varios músculos y ligamentos incertados en su superficie interna. Aquí se plantea la hipótesis que el disco abdominal presenta dos mecanismos para fijarse al sustrato: fricción y succion. El tama o y las áreas de inserción de los músculos en el disco abdominal sugieren que el mecanismo de succión es pasivo, o sea, sin la acción constante de los músculos.

Palabras clave: *Atelopus*, renacuajos, disco abdominal, adaptaciones reofílicos, adhesión al sustrato.

Abstract
The abdominal disc of *Atelopus* tadpole is a complex structure, which includes three well-defined regions with several muscles and ligaments attached to its internal surface. It is hypothesized that the disc has two mechanisms of attachment to the substrate, namely, friction and suction. The size and insertion points of the muscles and ligaments of the disc suggests that the suction mechanism works passively, without the constant action of the muscles.

Key words: *Atelopus*, Gastromyzophorus Tadpole, Abdominal Disc, Stream Adaptations, Attachment.

Introduction

Tadpoles having a ventral postoral abdominal disc (gastromyzophorus) are known in only four taxa: *Amolops* (Ranidae), *Atelophryniscus chrysophorus* (Bufonidae), *Atelopus* (Bufonidae), and *Rana sauteri* (Ranidae). All these taxa live in torrential streams with the ventral disc seeming to be an adaptation for that environment. Of these taxa, only the ventral disc of *Amolops* has been studied in detail (Annandale and Hora, 1922; Noble 1929; Hora 1932; Bhaduri 1935).

The abdominal disc is one of the most conspicuous and specialized structure in the larvae of the genus *Atelopus*. Its function is, at least in part, to help the tadpole attach to the surfaces of rocks, pebbles, and logs in the streams in which they live (Starrett 1967; Duellman and Lynch, 1969). The way in which the disc functions is unknown. A close examination of the surface of the disc reveals the existence of a complex topography and well-defined regions identifiable by differences in colors and textures.

A detailed study of the internal and external anatomy of the disc of *Atelopus* was made in order to describe the different regions of the disc and to discuss their possible relationship to attachment.

Materials and methods

The species and developmental stages (Gosner, 1960) of the tadpoles (in brackets) studied as follows: for internal morphology, *Atelopus ebenoides marinkellei* sensu Cochran and Goin (1970) (IND-AN 5134) [34, 38, 41, 42 , 43], *A. farci* (ICN 15853) [28, 33, 34, 38, 41, 42, 44], *A. ingnescens* (KU 180276) [34, 41], *A. nicefori* [42, 44], and *A. subornatus* [25]. For external morphology, *A. ebenoides marinkellei* (IND-AN 5134) [32, 38, 41, 42], *A. farci* (ICN 15853) [40, 41], and *A. subornatus* (ICN 15852) [30,34]. In all cases, one individual per stage was studied. Internal and external anatomical descriptions were based on one tadpole of *A. ebenoides marinkellei* in Stage 34 and one in Stage 32 respectively.

External morphology of the disc was studied under dissecting microscope. One tadpole of *A. subornatus* (Stage 33) and *A. ignescens* (stage 36) was prepared for scanning electron microscopy following Postek et al. (1980). For internal morphology, histological sections were prepared following Wessner (1960) and stained with Masson's trichrome. Also, to study muscle and ligament insertions, the discs of two *A. ebenoides marinkellei* tadpoles (Stage 38) were removed with a sharp knife, and stained with an iodine solution following Bock and Shear (1972). Terminology for external oral morphology of tadpoles follows that of Altig (1970), musculature follows that of Sedra (1950) and Duellman and Trueb (1986), and internal and external anatomy of the disc that of Noble (1929). Acronyms used as follows: IND-AN = Instituto Nacional de los Recursos Renovables y del Ambiente, Colombia; ICN = Instituto de Ciencias Naturales, Bogota, Colombia; KU = Museum of Natural History, University of Kansas.

Results

External and internal anatomy of the disc

The abdominal disc in *Atelopus* larvae is ovoid (wider than long) and it extends about two thirds of the body length, starting at the posterior borders of the oral disc. The abdominal disc is cup-like, shallow, and with a slightly concave valley and steep walls (Figure 1A). Three main regions are evident on the disc (Figure 1A): the free rim, a pale intermediate region, and a central pigmented lobe region. Also, there is a rough area, the friction area, located on the surface of the disc between the free rim and the intermediate region.

The free rim is the most peripheral part of the disc. The walls of the disc extend from the body and form a flap-like, flexible free rim. The distal part of the free rim is bulbous while the proximal part is thin (Figure 2A).

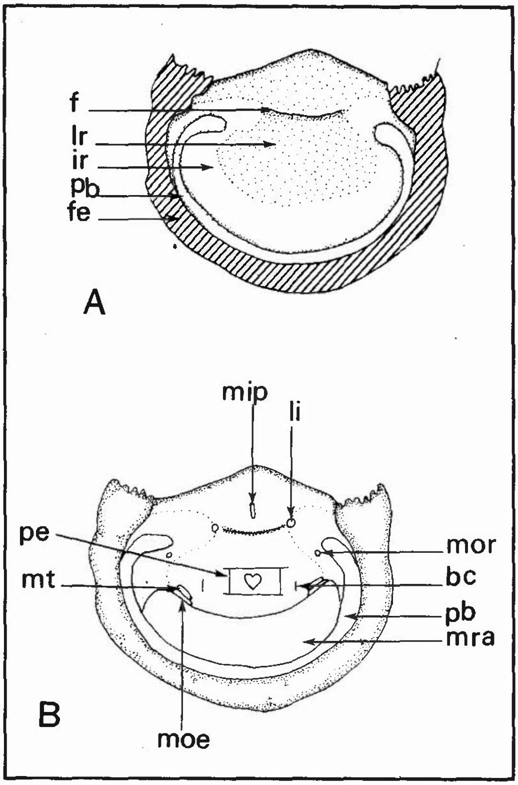

Figure 1. Diagram showing (A) the different regions of the disc of *Atelopus* determined on the basis of histology and external morphology. (B) the insertion points of the muscles, ligaments, and membranes into the abdominal disc. bc = branchial chamber wall; f = fold; fe = free edge; ir = intermediate region; li = ligaments; lr = lobe region; mip = m. intermandibularis posterior; moe = ramus of the m. obliquus externus; mor = ramus of the m. orbitohyoideus; mra = m. rectus abdominus; mt = ramus of the m. transversus; pb = friction area; pe = pericardia..

The distal part of the free rim is ventrally oriented at the anterior and posterior parts of the disc and laterally oriented at the middle section. At the most anterior part of the disc, the edge that contacts the lower lip widens (Figure 1A), but a free rim is not evident.

Histologically, the free rim is heterogeneous. Most of its bulbous distal part contains no cells or fibers while its proximal part always present loosely-disorganized fibers as well as few mesenchyme cells (Figure 2A). As a consequence, externally the distal part of the free rim is translucent while the proximal part pale. The cell-free distal part of the bulbous may be a lymphatic sinus or an artifact seen frequently in formaldehyde-preserved specimens. Presently, that is unknown. The free rim presents keratinized stratified squamosal epithelium which is two cells thick (the external layer flat and the internal cubic) at the lateral and dorsal surfaces, three cells thick at the ventral surface of the bulbous part, and four cells thick (or more) at the ventral surface of the proximal part.

The intermediate region is located between the free rim and the lobe region (Figure 1A). This region is a wide, crescent-shaped band with its anterior endings slightly directed medially. Externally, the intermediate region is unpigmented and paler than the free rim, except at its proximal part.

Histologically, the intermediate region is the area of the disc underlain by a pad of connective tissue (Figure 2B). The pad is thick and lies dorsal to the epithelium. Inside the pad there are sparse, mostly dorsoventrally oriented, spindle-shaped cells and fibers forming a loose network. The pad of connective tissue is surrounded by a single-cell membrane except at its lateral edge where such membrane is absent allowing some of the mesenchyme cells of the pad to enter the proximal part of the free rim (Figures 2A and 2B). The intermediate region has keratinized stratified squamosal epithelium two cells thick (the external layer flat and the internal cylindrical).

The lobe region is the central and most anterior part of the disc (Figure 1A). Externally, the lobe region is the only pigmented area of the disc, although frequently some pigment can be seen at the most anterior part of the free rim. The lobe region is divided into an anterior and a posterior lobes by a transverse fold of skin (Figure 1A).

Most of the muscles and ligaments of the disc are inserted into the lobe region, but no other specialized structure underlies that region. This area presents keratinized stratified squamosal epithelium (Figures 3A and 3C) which is two to three layers thick (the internal layers with large cubic cells, and the external with flat cells) dorsally bordered by a thin layer of densely-packed fibers of connective tissue. The transverse fold that divides the lobe region is formed by the insertion of two ligaments on the dorsal surface of the lobe's epithelium (Figure 3B). The walls of the transverse fold present a high concentration of glands.

Part of the epithelium of the intermediate region is modified to form a rough area, the friction area, which is mostly located at the periphery of the intermediate region but with a small part entering the free rim (Figure 2A). The friction area is narrow and crescent-shaped with its anterior ends slightly expanded and medially directed (Figure 1A). The friction area is recognized externally from other areas because it is the palest region of the disc.

The friction area is composed of minuscule protuberances arranged next to each other. The friction area is 10 to 15 protuberances wide (Figures 2C and 2D) except at the anterior edges, where it is much wider. Each protuberance is one cell wide and it is formed by the piling of cells on top of each other. At the base of each protuberance there is a large cell (sometimes itself protruding through the surface) with larger and paler nuclei than the other cells of the epithelium (Figure 2C). The most external cells of the protuberance are more pointed than the internal ones and are coated by several layers (up to five) of packed keratin. Not all protuberances are equally tall or composed of the same number of cells.

Muscles and ligaments

There are several small to medium size muscles, ligaments, and membranes inserted on various parts of the disc. No muscles are inserted on the free rim (Figure 1B).

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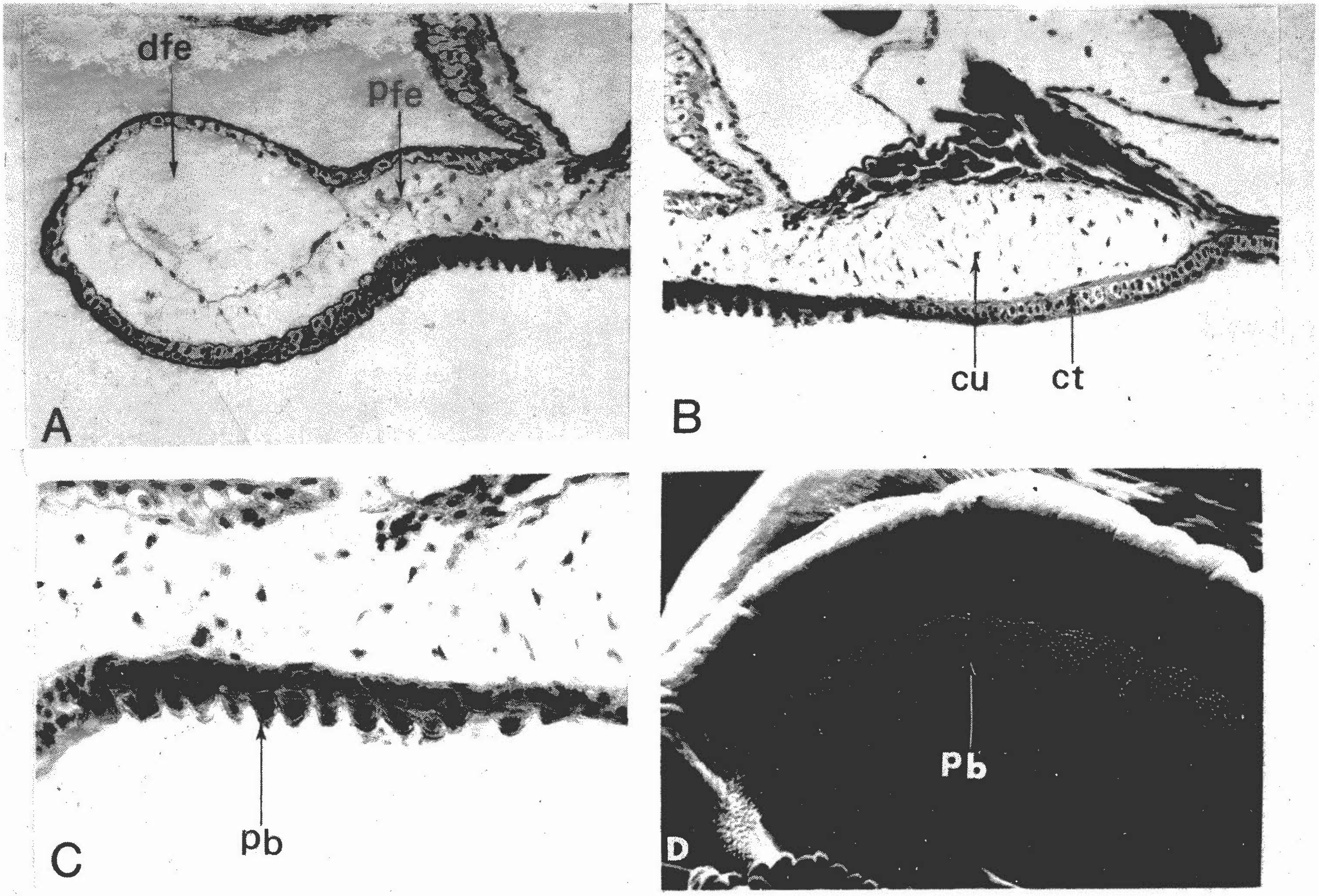

Figure 2. Transverse sections of different regions of the disc of *Atelopus farci*. (A) free edge. (B) intermediate region. (C) friction area. (D) S.E.M. photo of friction area of *A. ignescens*. ct = layer of connective tissue; cu = cushion; dfe = distal part of free edge; pb = fricion area; pfe = proximal part of free edge.

The muscles inserted on the lobe region and the pad of connective tissue underlying the intermediate region are the m. intermandibularis posterior, a ramus of the m. orbitohyoideus, a ramus of the m. obliquus externus, a ramus of the m. transversus, and the m. rectus abdominis. Also, one pair of ligaments plus the walls of the pericardium and branchial chambers are attached to the lobe region.

The two halves of the m. intermandibularis posterior extend with a gentle slope in a dorsoanterior-ventroposterior direction. Each originates anteriorly at the posteromedial part of Meckel's cartilage and fans out posteriorly to form a wide insertion on the layer of connective tissue which underlies the epithelium of the anterior part of the anterior lobe (Figure 3A). At their insertion, both sides of the m. intermandibularis posterior are separated by a very narrow gap. The contraction of this muscle would mostly pull the epithelium of the anterior lobe anteriorly and only slightly raise it.

There are two ligaments slightly posterior and lateral to the m. intermandibularis posterior. Each one originates dorsally, on the ventral side of the ceratohyal and insert ventrally on the anterolateral corners of the fold of skin which separates the anterior and posterior lobes. Before the insertion the ligaments fan out (Figure 3B). The formation of the fold of skin which divides the lobe region is most probably related to the insertion of those ligaments on the disc surface.

At the level of the anterior part of the m. subhyoideus and next to the point where the m. suspesorium hyoideus inserts on the ceratohyal, a very small ramus of the m. orbitohyoideus (Fig. 3C) branches off. That ramus first runs ventrally, passing lateral to the m. suspensorium hyoideus and, once it reaches the ventral part of the hyoid plate, then runs posteriorly (in contact with the ventral surface of the m. subhyoideus) and finally, after a short distance, runs ventrally to insert on the pad of connective tissue of the intermediate region. However, before inserting, the ramus of the m. orbitohyoideus splits in two, one branch inserting slightly anterior to the other.

Posterior to the m. subhyoideus on the posterior lobe, the walls of the pericardium (except its most anterior part) and branchial chambers attach to the disc (Figure 3D).

Immediately posterior to the heart, a lateral portion of the m. rectus abdominis and the anterior parts of a branch of the m. transversus and m. obliquus externus insert (next to each other) into the pad of connective tissue of the intermediate region (Figure 4A). Anteriorly, those three muscles are indistinguishable from each other. However, slightly posteriorly, part of the m. transversus and m. obliquus externus can be seen inserting into the lobe region right at the base of the pad of connnective tissue (Figure 4B) and their fibers start directing dorsoposteriorly until they join the main body of the m. obliquus externus and the more internal m. transversus. The lateral portion of the m. rectus abdominis extends posteriorly and slightly laterally until it finally merges with the main body of the muscle. Anteriorly the lateral portion of the m. rectus abdominis is flat, whereas posteriorly it has a pyramidal shape.

The ventral branch of the m. transversus and m. obliquus externus separates the lateral extension of the m. rectus abdominis from the m. sternohyoideus. However, at the posterior part of the disc (near where the pad of connective tissue extends completely across the tadpole's body), the branch of the m. transversus disappears and the m. sternoyoideus merges into the m. rectus abdominis, which already shows some fibers inserted medially into the sternohyoideus junction (Figure 4C).

Discussion

The anatomy of the abdominal disc of *Atelopus* suggests that the attachment of the larvae to the substrate (rocks, logs, leaves) is

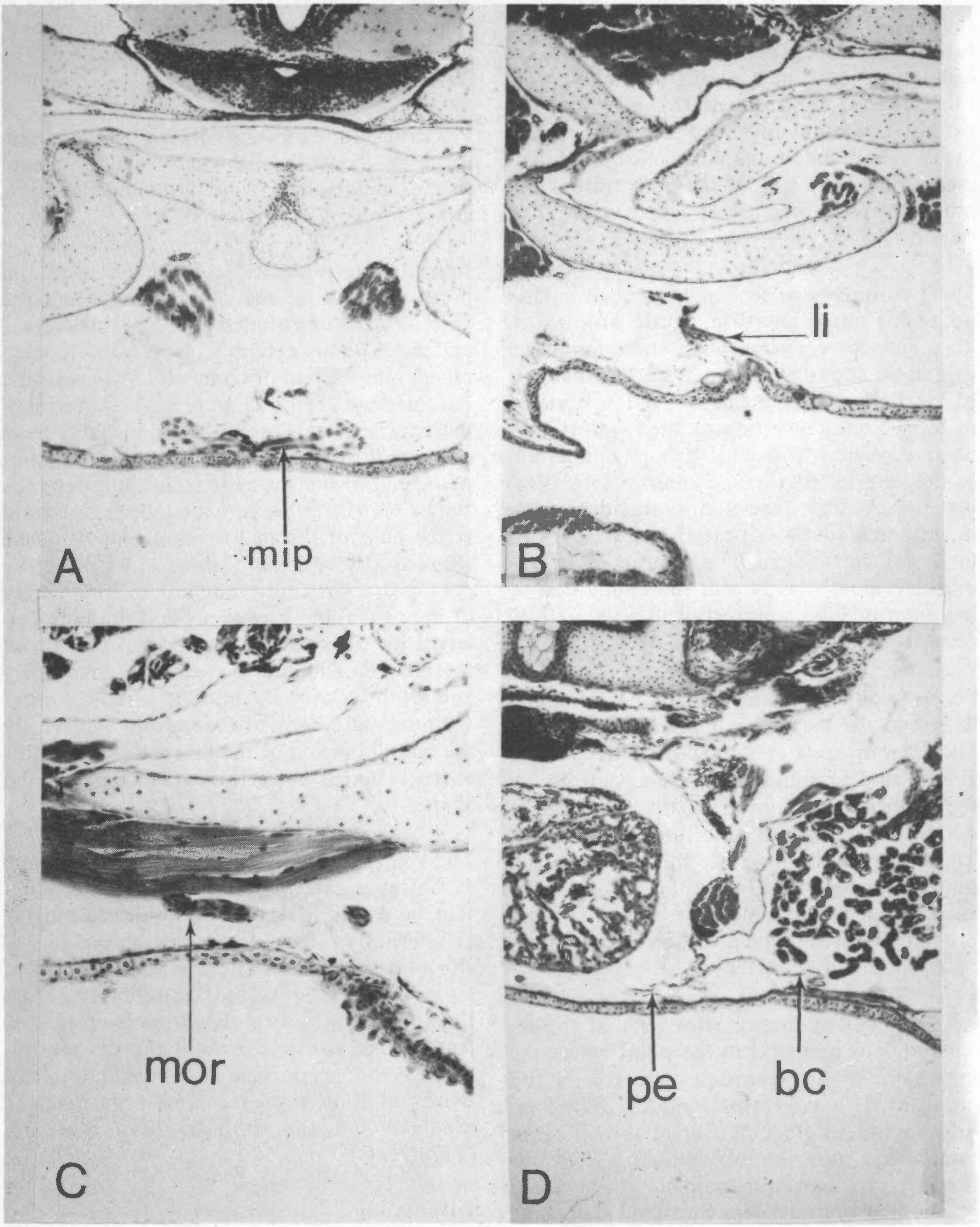

Figure 3. Transverse section through various muscles, ligaments and membranes attached to the abdominal disc in *Atelopus farci.* (A) m. intermandibularis. (B) Ligament. (C) Ramus of the m. orbitohyoideus. (D) pericardium and branchial chamber wall. bc = branchial chamber wall; li = ligament; mip = m. intermandibularis posterior; mor = ramus of the m. orbitohyoideus; pe = pericardia.

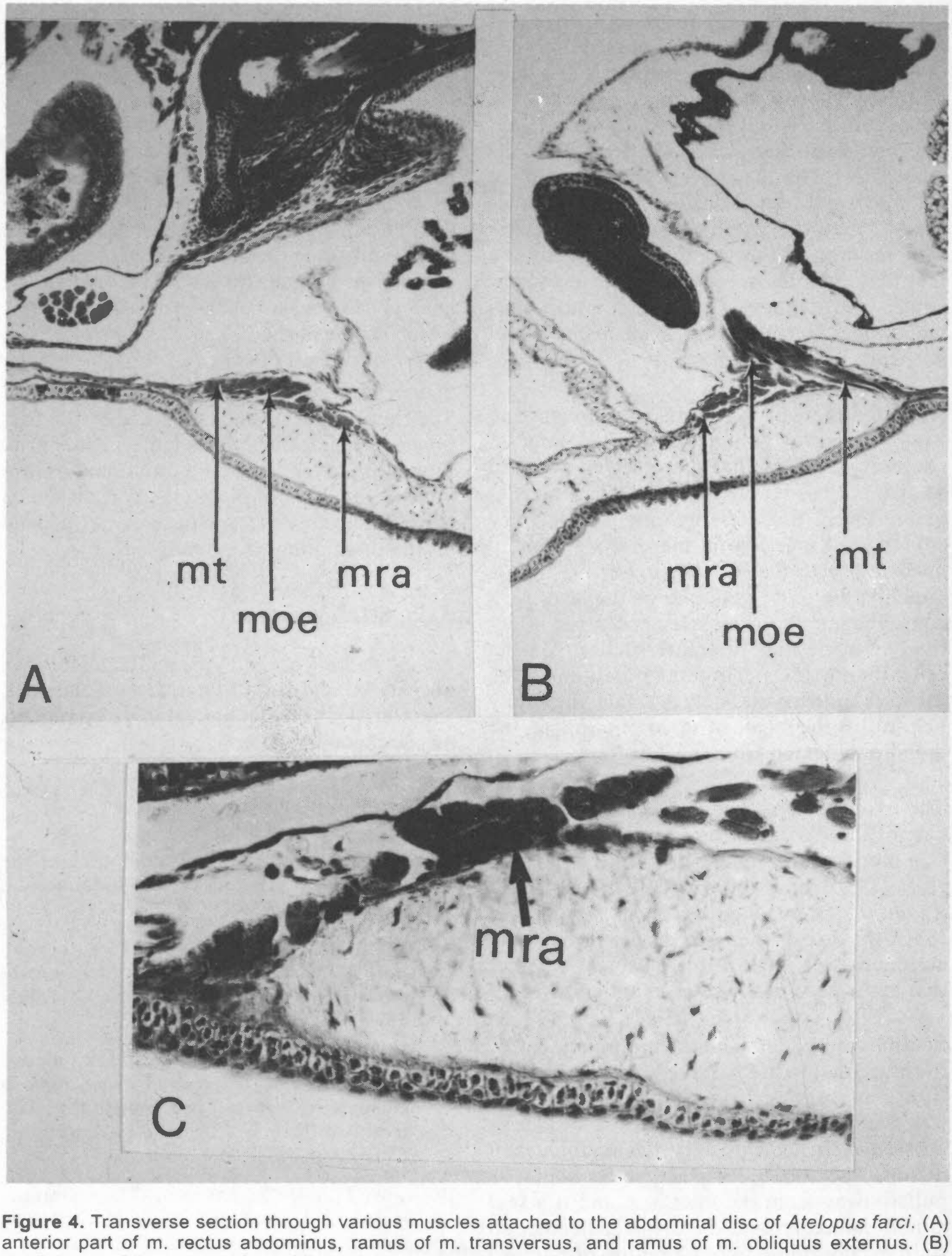

Figure 4. Transverse section through various muscles attached to the abdominal disc of *Atelopus farci*. (A) anterior part of m. rectus abdominus, ramus of m. transversus, and ramus of m. obliquus externus. (B) medial part of the ramus of m. obliquus externus, m. rectus abdominus, and ramus of m. transversus. (C) merging of the m. sternohyoideus with the main body of the m. rectus abdominus. moe = ramus of the m. obliquus externus; mra = m. rectus abdominus; mt = ramus of the m. transversus.

accomplished by means of two different mechanism, namely, suction and friction. The existence of a suction mechanism is also suggested by the cup shape of the disc and by the observation (Duellman and Lynch, 1969, Starrett 1986) that these tadpoles remain attached to rocks that have been lifted above the water and turned upsidedown. The existence of a friction mechanism is suggested by the presence of the friction area and by the fact that tadpoles are found attached to substrates with textures (i.e., rough and porous wood) on which a suction mechanisms would not work.

Although the way suction is accomplished in *Atelopus* larvae is not known, the internal anatomy suggests that the muscles attached to disc are not ivolved in the suction mechanism. These muscles are few, small, very angled, and incerted on the disc's periphery on or close to the pad of connective tissue, which is the most rigid part of the disc. As a consequence, these muscles could not effectively lift the disc to create suction. On the other hand, the peripheral position of the muscles' incertions suggests that they may help to lift the edges of the disc allowing the tadpole to detach from the substrate.

The two ligaments inserted at a more central part of the disc, may have a role in the suction mechanism. Annandale and Hora (1922), Hora (1930), and Noble (1929) argued that in *Amolops,* the attached ligaments would help to lift the disc as the body arches through the contraction of some of the muscles. Whether that is the case in *Atelopus* is not known.

Suction can occur without the involment of the musculature in a passive way (passive suction), analogous to a suction cup. Vogel (1988:286) said that «if a flexible surface is pressed against a rigid one, if some force then distorts the flexible surface so its center is pulled away from the interface, and if a seal is maintained around the displaced center so fluid cannot move into the region of reduced pressure, then the external atmosphere will push the surfaces together». Whether this is the case in *Atelopus* is not known. However, the fact that the tadpoles of *Atelopus* are capable of eating while crawling on the substrate without detaching (pers. obs.) is consistent with the passive suction hypothesis. That is because if there are no muscles involved in the attachment mechanism, there is no need for a simultaneous complex coordinated action of several muscles, some to attach and some to eat. The movement of the tadpole on the substrate's surface would be analogous to what occurs with suction cups, which can be pushed along the substrate (i.e., glass) while still attached to it.

The friction mecanism is provided by the friction area which allow the tadpole a better grip. In nature, *Atelopus* tadpoles are found of logs both facing and not facing the current which suggest that this mecanism does not depend on the orientation of the tadpole.

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